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TITLE: Stroma-Based Prognosticators Incorporating Differences between African and European Americans

PRINCIPAL INVESTIGATOR: Michael McClelland

CONTRACTING ORGANIZATION: University of California Irvine
Irvine, CA 92697

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Michael McClelland, Dan Mercola, Mike Lilly

E-Mail: mmcclell@uci.edu

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13. SUPPLEMENTARY NOTES

14. ABSTRACT. Our objective is to identify and validate RNA prognosticators for prostate cancer in European Americans (EA) and African Americans (AA), and port these to DNA methylation and protein-based prognosticators, where possible. The unique aspects of this project are that it uses tumor-adjacent stroma as a source of markers, and uses standard Formalin-fixed Paraffin-embedded (FFPE) tissue, which is a challenge for molecular biology. In the first year, we profiled RNA by multiplex array cards and found that only half the samples were of adequate quality. Even fewer DNA samples from the same patients were amenable to bisulfite sequencing of more than a few genes. Exploiting the recent three-fold reduction in the cost of sequencing per read, we developed oligo capture sequencing methods to extract global RNA expression data, and antibody methods to capture genome-wide DNA methylation data. These protocols work on samples over almost the entire range of quality and yield, which would otherwise be lost to the study. Many of these samples are all the more precious owing to longer follow-up including samples from AA patients. We used Gleason Score, chemical relapse status, and approximate age, to match patients. We have now obtained comprehensive expression data from 18 AA, and 27 matched EA, and genome-wide methylation data from 13 and 7 of the same patients. Awaiting analysis are all the required additional pairs of AA and EA patients.

15. SUBJECT TERMS
Prostate, Stroma, Biomarkers, African American, DNA methylation, RNA, Prognosis.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. Introduction…….

Every prostate tumor is heterogeneous, with DNA mutations and copy number differences. This makes the identification of dependable biomarkers in tumors more difficult. In contrast, there are fewer mutations in the tumor-adjacent stroma, which nevertheless has many RNA expression changes relative to tumor-free stroma, presumably due to interactions with the tumor. The abundant tumor-adjacent stroma is a potential source of prognosticators for prostate cancer progression.

The epidemiology of the disease is different in African Americans (AA) and European Americans (EA) and we and others have found RNA expression changes that correlate with race. These differences in gene expression may be associated with differences in progression. We proposed to investigate potentially prognostic biomarkers in both AA and EA patients, with the possibility that best prognosticators in each race may not be the same. We also proposed to study DNA methylation differences in Formalin-fixed paraffin-embedded (FFPE) samples (the material utilized in Pathology). FFPE samples are a more dependable source of DNA than of RNA. An accurate prediction of a high risk of relapse following surgery may be the basis for electing immediate adjuvant therapy following surgery. An improved prognosticator for AA patients would immensely benefit this disproportionally highly affected population.

The major challenge to validating the strategy is that it requires retrospective study of old FFPE samples, whereas after validation, biomarkers would be used on fresh FFPE samples, which are provide higher quality RNA and DNA. Thus, gathering data for validation is more of a technical challenge than implementation after validation. We have spent a considerable portion of our effort on developing a way to side-step technical issue, with great success.

2. Keywords…….

Prostate, Stroma, Prognosis, Biomarkers, African American, DNA methylation, RNA.

3. Accomplishments……..……...

- **What were the major goals of the project?**

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1. Validation of the stroma-based prognostic classifier(s) for both African and European Americans.		
Major Task 1: Obtain and process FFPE tissue for RNA and DNA isolation and identify prognosticators from training set.	months	Status

Subtask 1: Regulatory review and approval processes, including local Institutional Review Board (IRB) and DoD Human Research Protection Office (HRPO).	1-3	100%
Subtask 2: Select appropriate exon-exon spanning primers for qPCR for the 96 genes to be surveyed.	1-6	100%
Subtask 3: Validate primer quality using bulk RNA.	4-6	100%
Subtask 4: Identify the first set of 60 suitable African American (AA) and 60 clinically matched European American (EA) samples with recurrence status and at least five years of follow-up. Training set.	1-6	100%
Subtask 5: Prepare both RNA and DNA from dissected slides. First 60 pairs of cases. Training set.	3-9	100%
Subtask 6: Validate RNA quality using control primer pairs.	6-9	100%
Subtask 7: Perform initial data collection on 96 validated primer pairs and 120 validated RNAs. Training set.	9-12	50%
Subtask 8: Construct prognosticators for recurrence in AA and EA separately, and in AA+EA combined, with and without clinical parameters. Training set.	12-15	50%
Milestone Achieved: RNA-based prognosticator for recurrence refined based on training set. Retargeted to RNAseq		
Major Task 2: Perform blinded randomized test.	months	Locations
Subtask 1: Identify the second set of 60 different AA and 60 clinically matched EA samples with recurrence status and at least five years of follow-up. Include a blinding and randomization step. Testing (validation) set. *Retargeted to RNAseq*	6-12	100%
Subtask 2: Prepare both RNA and DNA from dissected slides. Second 60 pairs of cases. Testing/validation set.	12-18	10%
Subtask 3: Validate RNA quality using control primer pairs.	15-18	10%
Subtask 4: Perform data collection on validated primers and validated RNAs. Testing/validation set.	18-24	20%
Subtask 5: Assess performance of prognosticators for recurrence in AA and EA separately, and in AA+EA combined, with and without clinical parameters, using the outside statistician. Testing/validation set.	24-27	To be done
Milestone Achieved: Identification of prognostic power of the refined prognosticator in a randomized blinded trial.		
Specific Aim 2: Test for DNA methylation differences near genes that have prognostic RNA expression differences.		
Major Task 1: DNA quality assessment, PCR optimization, sequencing.		
Subtask 1: Determine which regions near each of the 96 genes in Aim 1 (including control genes) show evidence of differential methylation in our reference set of whole genome methylation profiling. *Retargeted to all genes instead of just 96*	1-6	100%
Subtask 2: Design unique primer pairs to the relevant regions, based on the sequence after bisulfite treatment.	6-12	10%

Subtask 3: Determine which genes in Aim 1 have a prognostic or race-enriched expression profile after training.	12-15	20%
Subtask 4: Test up to 50 primer pairs on bulk bisulfite treated DNA. Then multiplex and adjust as necessary.	15-18	To be done
Subtask 5: Determine quality of DNAs from samples that were prepared in Aim 1, Major task 1, Subtask 5 using control primer pairs.	18-21	To be done
Subtask 6: Bisulfite treatment of DNAs that pass the above test and test again for bisulfite treatment quality with control primers.	21-24	To be done
Subtask 7: Screen bisulfite treated DNAs that pass the above test using up to 50 primer pairs arranged in ~10 multiplexes. PCR to add index primers.	24-30	To be done
Subtask 8: Pool all PCR reactions, clean, and apply to an Illumina sequencer for 2 x 150 PE sequencing	30	To be done
Subtask 9: Deduplicate, assemble each target region, and determine the frequency and allele distribution of DNA methylation in each case.	33-34	To be done
Milestone Achieved: Determination of DNA methylation differences in genes of known prognostic or race-enriched expression profile.		
Specific Aim 3: Test for congruence of prognostic RNA expression differences with protein expression differences.		
Major Task 1: Obtain and process FFPE tissue for protein expression analysis.	months	Locations
Subtask 1: Determine which of the 96 candidate proteins from Aim 1 shows evidence of expression in the Human Protein Atlas.	1-6	100%
Subtask 2: Identify suitable antibodies for the subset of proteins that pass the filter above.	6-12	100%
Subtask 3: Determine which genes in Aim 1 have a prognostic or race-enriched expression profile after training. (same as Aim 2, Major Task 1, Subtask 3).	12-15	To be done
Subtask 4: Test antibodies on waste sections of TMA for performance. Compare to control antibodies.	15-18	To be done
Subtask 5: Perform IHC on TMAs and scan with up to 10 functional antibodies.	18-33	To be done
Subtask 6: Perform visual data analysis and quantitation.	21-36	To be done
Subtask 7: Assess which proteins have an antibody staining distribution between patients indicative of a correlation with RNA expression, with prognostics, and/or with race.	24-36	To be done
Milestone Achieved: Identification of proteins whose expression is of prognostic value and/or varies between races.		

- **What was accomplished under these goals?**

Aim 1, major task 1, subtask 1 (regulatory review), *2* (primer design), and *3* (validate primer quality), were completed as planned in year one, using the methods in

the proposal. Other early tasks completed were *Aim 3 subtask 1* (identify genes expressed in the prostate at the protein level) and *subtask 2* (identify suitable antibodies).

Aim 1, major task 1, subtask 4 (identifying pairs of patients) is critical because the quality of the metadata and matching AA and EA patients will strongly influence the results. For this task we diligently accumulated 70 precious AA samples with good follow-up data from our partner at the Medical University of South Carolina. We added over 150 additional AA and EA samples to our collection in the first year and have now added 129 more in our second year. This greatly exceeded our goal but allows us to match patients even better than planned. Of particular note are our efforts in the second year to also collect untreated cystoprostatectomies without prostate cancer, warm autopsy, and TURPs obtained from debulking of BPH. These efforts will continue in year three. **Table 1** shows the overall patient samples we have now accumulated for the project.

Table 1: 129 new patient samples added in second year

		Race or Ethnicity							
Gleason Score	**Total cases**	**AA**	**EA**	**Asian**	**Hispanic**	**Unknown**	**Relapse**	**Non-Relapse**	**Pending Review**
G5	5		5				2	3	
G6	180	28	144	3	1	4	142	38	7
G7	266	87	113	29	7	30	268	159	33
G8	65	29	36				41	23	2
G9	90	16	48	26			74	16	5
Unknown	32		8	24					
Non-tumor	24	1	19	4					

In *Aim 1, major task 1, Subtask 5*, we prepared both RNA and DNA from the same sample for 40 AA and 70 EA, including samples that could meet the criteria for *Aim 1, major task 2, subtask 1*. We carefully mark pure tumor-adjacent stroma, and take multiple 1 mm punches. This is very different from taking out a big chunk from an FFPE block. We then validated quality in *Aim 1, subtask 6*. Some samples produced adequate quality. However, some samples did not. Some of the older FFPE samples with long-term follow-up, in particular, do not have an RNA integrity number (RIN) or yield (or both) sufficient for use in multiple PCR reactions on array cards (Aim 1). Similarly, although we can simultaneously obtain hundreds of nanograms of DNA from FFPE punches, this is often of sufficient quality for just a few bisulfite sequencing reactions, and not suitable for massively in parallel PCR of the majority of candidate DNA regions (Aim 2). The older samples are often the most valuable because they generally have the most clinical follow-up data and some are from minority AA.

Nevertheless, as required by the proposal, during the second year, we expanded our array card dataset and then iteratively trimmed it for poor performing oligos and samples. Having complete this Task as laid out in the grant proposal, we obtained a positive predictive value of 0.75, which is encouraging, but also we lost over half of our

patients to the inadequate quality of some FFPE samples, particularly AA samples because their relative rarity often required us to use older samples. This precluded determining if performance was better on a racial basis for a subset of markers.

Rather than make any change our objectives, in the first year we began to converted these samples into libraries, and sequenced them. In the case of RNA of low quality and/or yield, we have used the "Access" method of Illumina, to perform sequence-specific **capture** of coding **RNA**. This has allowed us to establish that we have enough complexity remaining across each gene to be able to do expression analysis for these valuable patients with samples of RIN at or below 2.5, often considered poor quality, and using a mass of 100 ng, far less than could be used to examine our genes using array cards.

The ability of the HiSeq 4000 to obtain about three times as many reads as the HiSeq2500, at the same price, means we can stay on track, though sample processing is more time consuming. We have now greatly expanded the number of samples processed through RNA library production, oligo capture, and sequencing (**Table 2**). We obtain tens of millions of mapped deduplicated reads per sample, while using only 5% of a sequencing lane per sample. Most of our precious lower quality samples have been rescued in this way. The RNA libraries give us expression information on all our candidate genes on samples that cannot be use array cards and at a price comparable to the approach we were taking with array cards. Thus, our goal of prognosticator validation is not affected.

Table 2: Tumor-adjacent stroma gene regulation data

	Array cards	RNA expression analysis	Whole genome methylation analysis
AA tumor	6	18	13
EA tumor	39	27	7
AA CAFs	1	4	4
EA CAFs		4	4
Non-cancer		3	

In the training phase we are using FFPE blocks that are almost *all a lot older than the patient samples that will be used for prognostics*. Thus, there is no concern that when the test has been validated that it could be readily applied to fresher FFPE samples directly on gene-specific assays, such as array cards. That ultimate goal remains unchanged.

An advantage of targeting tumor-adjacent stroma has become evident during the project. If we were confronting these circumstances with tumor tissue, it would be more difficult to resolve: We have also been collecting tumor when punching out tumor-adjacent stroma, and archiving it, but pure tumor punches are almost impossible to obtain in many blocks. Indeed, in addition to about 20% of blocks being tumor-negative during our search for tumor-adjacent stroma (which adds to the challenges), an additional 20% have only just enough tumor to be able to identify tumor-adjacent stroma, but not enough pure tumor for collection.

Aim 1, major task 2. This task identified the additional samples needed for validation. We have exceeded the collection goals. The validation goals are going to require the collection of more RNAseq data, which is ongoing.

Aim 2, is focused on DNA methylation in tumor. We identified regions near genes of interest that are differentially methylated in prostate stroma. In the case of DNA of low quality and/or yield, massively in-parallel targeted bisulfite sequencing proved to be out of the question, and we do not want to lose these samples because they tend to be the older and most informative ones.

Making a virtue of necessity, we have used an approach that works on these samples: the antibody capture methylation profiling described in the grant. Almost every sample has proven good enough for methylation capture, even when it was too poor for bisulfite sequencing. We were able to obtain tens of millions of deduplicated mapped reads both using methylCpG and unmethyl-CpG antibody and identify thousands of high confidence differentially methylated locations (**Table 2**).

We are focused on testing our prognosticator by the best available approach. A new kit by Illumina has just been released that uses oligo capture for targeted bisulfite sequencing. This strategy focuses on predefined regions of the genome. We have begun to explore this strategy with a couple of sequencing reactions and find it works with high quality abundant DNA, but not DNA of the quality we often obtain from old FFPE blocks. Thus, for now, our more agnostic approach, surveying the whole genome, is of most utility because it has yielded differentially methylated sites in or near tens of genes of interest that are not present in the captured subset in the commercial kit.

One particularly interesting additional asset has become available to the project, and at no cost to the project. A colleague (Xi) has created over 100 lines of Cancer-associated fibroblasts from adjacent stroma in about 50 AA and 50 EA patients. He and another collaborator (Agrawal), in consultation with us, examined 30 secreted cytokines in 20 AA and 20 EA cultured lines and found significant differences between AA and EA in 20 of these important secreted signaling factors, indicating differences are preserved in culture. On this basis, we have performed a limited set of RNAseq and Methyl-capture on a few of these lines (**Table 2**), with the idea that the prognostic genes we identify in this project will then have actual cell lines upon which the community will be able to perform functional tests for therapeutic targets. In addition to using the data in the current project, we expect to develop this asset further in future grant applications.

- **What opportunities for training and professional development has the project provided?**

Farah Rahmatpanah, the primary bench researcher, has attended a total of five conferences, to date, and presented posters.

In addition to the people providing paid effort, other people have participated in this project without pay, in exchange for training. Those that were in training in the second year included:

- Sameer Kapadia (Undergraduate) from 2016-2017.
- Parsa Rahmantpanah (Undergraduate) 2015-2017.
- Hannah Nguyen (Undergraduate). June 2017-present.
- Majed Alnabulsi (Visiting MD). 2017.

The following people donated time and resources to pay for the creation of cultured cancer-associated fibroblasts (CAFs) and the Luminex assays that proved AA and EA cancer stroma are different, even *in vitro*. We are now including these CAFs in our biomarker screening.

- Xiaolin Zi, Professor, Department of Urology.
- Anshu Agrawal Assistant Adjunct Professor, Department of Medicine.
- Sudhanshu Agrawal, Specialist, Department of Medicine, Immunology.

- **How were the results disseminated to communities of interest?**

Outreach:

- Two community talks at fundraisers for Prostate Cancer Research Foundation.
- Lecture on Prostate CA at a Summer Cancer Research Internship, Senior High School students, 2017
- Fellowship core lectures, Systemic treatment of PCa, HemOnc Fellows. 2017
- Prostate cancer genomics. Postdoctoral fellows. 2016. Two lectures
- PCa Immunotherapy, seminar, MUSC, 2017
- PCa and translational research opportunities, seminar, MUSC 2017
- Lecture to the MUSC HCC Citizens' Advisory Board

- **What do you plan to do during the next reporting period to accomplish the goals?**

Subtask 8 of Aim 1 is the heart of the project and given the low RIN we obtained from many old samples, it was our greatest challenge. Having changed to oligo capture for RNAseq, and global antibody capture for methylated DNA, our success rate for old samples has more than doubled.

The vastly greater additional genome-wide expression and methylation information we obtain from the lower quality samples will allow us to iteratively prioritize certain genes over others for RT-PCR. Knowing which genes are most closely related to prognosis in AA and EA will be required in order to focus on the best methylation candidates in Aim 2 and the best antibodies in Aim 3.

Extraordinary strides in sensitivity and throughput in nucleic acid quantitation are occurring. These advances may allow us to use old FFPE samples that even with our new approaches, are currently hopelessly degraded and low abundance. We are keeping these samples for when technology allows their use.

4. Impact……..……

- **What was the impact on the development of the principal discipline(s) of the project?**

We have made what may be a major contribution to the problem of obtaining high quality data from archived FFPE samples that were beyond the capabilities of array cards. In a slow progressing disease, like prostate cancer, where samples of interest for training and validation are necessarily old, this improvement is pivotal to future success of RNA and DNA biomarkers, which then lead to protein biomarkers. The data we have

obtained and will continue to obtain in the next reporting period, is a potential goldmine for the community to validate *their markers* on data from these same samples with long-term clinical follow-up.

- **What was the impact on other disciplines?**

The ability to screen very old FFPE samples is especially of interest for prostate cancer, but this is not the only disease where such ability will be important.

The unmethyl-CpG antibody has been little used by others, and we find it is exquisitely specific to CpG islands, whereas the methyl-CpG antibody assays methylation in a lot of the genome due to a requirement for fewer methylated-C in a DNA fragment for successful capture. The difference in the spectrum of capture of the mCpG and unmethylated-CpG antibodies may be exploitable in any system where differential methylation is of interest. The fact that most old FFPE samples can yield enough DNA for only a very limited set of bisulfite sequencing reactions, means that antibody capture methods probably have a long-term future.

- **What was the impact on technology transfer?**

  - Our results from highly degrades FFPE samples may be patentable.
  - We will protect the prognosticators. However, we need to process more samples, first.

- **What was the impact on society beyond science and technology?**

Nothing to report.

5. Changes/Problems……

- **Changes in approach and reasons for change**

The goals remain unchanged. However, as reported last year, so many samples were of inadequate quality for the array cards that we explored using oligo capture and sequencing for RNA, and antibody capture for DNA methylation. In the second years, this has been remarkably successful; succeeding with far less RNA and of lower quality than the array cards required.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

As reported last year, the quality in the oldest FFPE samples was a problem, but we have learned how to deal with it, as explained above, with only a moderate delay.

- **Changes that had a significant impact on expenditures**

As reported last year, the fall in the cost of Illumina sequencing on the HiSeq 4000 opened an opportunity for low quality samples that would otherwise have been

prohibitively expensive. This has allowed a greater fraction of our huge effort on processing clinical samples to ultimately result in data. Although downstream bioinformatics is more labor-intensive than planned, the much richer dataset is a precious boon, generating a unique resource.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  None

- **Significant changes in use or care of human subjects**

  None

- **Significant changes in use or care of vertebrate animals.**

  Not applicable

- **Significant changes in use of biohazards and/or select agents**

  None

6. Products……

- **Cumulative Publications, conference papers, and presentations**

Rahmatpanah, Farah, Zhenyu Jia, Bozhao Men, Parsa Rahmatpanah, Sepideh Madahian, Michael McClelland, and Dan Mercola. "*Methylation correlates with suppressed expression of immunomodulatory genes in the tumor-adjacent stroma of African American Prostate Cancer compared patients of European American ancestry.*" ASBMB meeting annual meeting (EB 2016), San Diego, CA, 2016.
http://www.fasebj.org/content/30/1_Supplement/1053.7?related-urls=yes&legid=fasebj;30/1_Supplement/1053.7

Lernhardt, W., Fiedler, F. Lasitschka, H. Kremling, F. Zinnhammer, F. Autschbach, D. Mercola, K. Schütze, J. Rassweiler. *Raman micro-spectroscopy: Potential for diagnosis and prediction of prostate cancer outcome.* Meeting of the EAU Section of Uro-Technology (ESUT), Athens, Greece. 2016.
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Rahmatpanah, Farah, Kathleen McGuire, Michael McClelland, Dan Mercola. *The use of whole genome methylation scanning to define genes preferentially suppressed in African American prostate Cancer*. The Science of Cancer Health Disparities in Racial/ Ethnic Minorities and the Medically Underserved. AACR meeting. Fort Lauderdale, FL. AACR program and proceeding. 2016.
http://cebp.aacrjournals.org/content/26/2_Supplement/B04

Lilly, Michael B. et al., *Circulating tumor (ct)-DNA profiling for potentially actionable targets in prostate cancer (PCa).* Meeting on Urological Cancers, Milan, Italy. 2016. http://www.eusupplements.europeanurology.com/article/S1569-9056(16)30376-1/pdf

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vanDraanen, J. M., Davidson, H. Bour-Jordan, L. Bowman-Carpio, D. Boyle, S. Dubinett, B. Gardner, J. Gardner, C. McFall, D. Mercola, T. Nakazono, S. Soares, H. Stoppler, M. Tempero, S. Vandenberg, Y.Y. Wan, S. Dry. *Assessing Researcher Needs for a Virtual Biobank.* Biopreservation and Biobanking. 2017:15:203-210.

Jia Arthur, Lee, Chung, McClelland, Michael, Mercola, Dan. *Tumor Microenvironment: Prospects for Diagnosis and Prognosis of Prostate Cancer Based on Changes in Tumor-Adjacent Stroma.* Precision Molecular Pathology of Prostate Cancer. 2017 (in press).

Website(s) or other Internet site(s)

None

Technologies or techniques

The use of complementary CpG antibodies, and demonstration of the ability to resurrect otherwise intractable samples with commercial oligo capture methods will be of wide interest as technology in upcoming manuscripts.

Inventions, patent applications, and/or licenses

Patents on the initial prognosticator were initiated prior to the award of the grant. Patent filings are planned once we reach the numbers of processed samples needed, which was scheduled for the end of the project.

Other Products

Sequencing data will be deposited in GEO once it is validated.

7. Participants & Other Collaborating Organizations……

- **What individuals have worked on the project?**

Name:	*Michael McClelland*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0003-1788-9347
Nearest person month worked:	*2*
Contribution to Project:	*Supervision, data analysis, writing*
Funding Support:	*Effort is 10% of NIH cap. Contributes about 10% more effort from UC Salary.*

Name:	*Dan Mercola*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	0000-0002-0281-9840
Nearest person month worked:	*2*
Contribution to Project:	*Supervision, data analysis, writing*
Funding Support:	*Effort is 10% of NIH cap. Contributes about 10% more effort from UC Salary.*

Name:	*Mike Lilly*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9412-8109
Nearest person month worked:	*1*
Contribution to Project:	*Management of AA sample acquisition*
Funding Support:	*Effort beyond the 1% budgeted (MUSC) is donated*

Name:	*Farah Rahmatpanah*
Project Role:	*Assistant Project Scientist*
Researcher Identifier (e.g. ORCID ID):	0000-0002-6158-1692
Nearest person month worked:	*6*
Contribution to Project:	*The primary bench scientist on the project*
Funding Support:	*Effort on this grant*

Name:	*Steffen Porwollik*

Project Role:	*Assistant Project Scientist*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9616-614
Nearest person month worked:	*1*
Contribution to Project:	*Data management*
Funding Support:	*Effort on this grant*

Name:	*Weiping Chu*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Sample processing.*
Funding Support:	*Effort on this grant*

Name:	*Mary Berkaw*
Project Role:	*Technologist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Sample acquisition and processing*
Funding Support:	*Effort on this grant (MUSC)*

Name:	*Anne Sawyer*
Project Role:	*Staff Research Associate II*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Sample acquisition. Sample logging*
Funding Support:	*Effort on this grant*

Name:	*Rachel Mendelson*
Project Role:	*Junior Specialist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0*

Contribution to Project:	*Programming*
Funding Support:	*0.1% Effort on this grant*

Name:	*Fred Long*
Project Role:	*Specialist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0*
Contribution to Project:	*Database management*
Funding Support:	*0.1% Effort on this grant*

Name:	*Zijin Wang*
Project Role:	*MS Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0*
Contribution to Project:	*Database management*
Funding Support:	*0.1% Effort on this grant*

Name:	*Yitong Wu*
Project Role:	*MS Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0*
Contribution to Project:	*Database management*
Funding Support:	*0.1% Effort on this grant*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

The following new grants are awarded.

1U54CA210963-01 (Salley) 09/01/17-08/31/22
South Carolina Cancer Disparities Research Center (SC CADRE).
This grant seeks to develop collaborative cancer research and treatment programs between MUSC and South Carolina State University (SCSU), to focus on cancer disparities. the initial funding period will involve several projects around racial disparities in prostate cancer.There is no overlap with the current project.

NIH R01AI044198-16 (Tan) 08/01/17-07/31/22
Mechanisms of temporal gene regulation in Chlamydia
Role: Co-investigator. 2% effort.
Major Goal: Systems biology of the infection cycle.

USDA (Andrews/McClelland) 02/01/17-12/31/20
Definition of Targets to Inhibit Salmonella Colonization of the Intestine
Role: Site Principal Investigator. 2% effort.
Major Goal: Pathways to bacterial colonization of the gut

USDA (Melotto/McClelland) 02/01/17-12/31/19
Mechanisms of *Salmonella* adaptation to the lettuce phyllosphere
Role: Site Principal Investigator. 2% effort.
Major Goal: Invasion genes for leaf colonization

The following grants have expired on schedule in the reporting year:

3U01CA086402-14S2 (I Thompson/Mercola). 07/01/14- 06/30/17
"San Antonio Center for Biomarkers of Risk for Prostate Cancer (SABOR)", a multisite study.

DOD CDMRP PC120465 (Mercola) 09/01/13-08/31/16
Validation of Biomarkers of the Tumor Microenvironment

USDA UF11033 (McClelland/Teplitski) 02/01/11-06/30/16
Salmonella interactions with tomatoes

UC system cancer grant (McClelland) 07/01/15-06/30/16
Exploiting the effect of bacteria on tumors

- **What other organizations were involved as partners?**
  Nothing to report

8. Special Reporting Requirements……
None

9. Appendices……
Links to works mentioned above:
http://www.fasebj.org/content/30/1_Supplement/1053.7?related-urls=yes&legid=fasebj;30/1_Supplement/1053.7
http://www.eusupplements.europeanurology.com/article/S1569-9056(16)15111-5/fulltext
http://cebp.aacrjournals.org/content/26/2_Supplement/B04
http://www.eusupplements.europeanurology.com/article/S1569-9056(16)30376-1/pdf
http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.5035
http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.6_suppl.149